PATENT COOPERATION TREATY

CORRECTED VERSION

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 004807-0009	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/CA2004/000006	International filing date *(day/month/year)* 13.01.2004	Priority date *(day/month/year)* 13.01.2003
International Patent Classification (IPC) or national classification and IPC C12N15/82, A01H5/00, A01H1/02, A01N37/06		
Applicant FLORISYS INC.et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of M sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16.02.2005	06.07.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - Gitschiner Str. 103 D-10958 Berlin Tel. +49 30 25901 - 0 Fax: +49 30 25901 - 840	Authorized Officer Schönwasser, D Telephone No. +49 30 25901-318

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/CA2004/000006

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-18	as originally filed

Sequence listings part of the description, Pages

1-4	received on 24.05.2004 with letter of 20.05.2004

Claims, Numbers

1-10, 18-22	received on 18.02.2005 with letter of 16.02.2005
11-17	received on 30.05.2005 with letter of 25.05.2005

Drawings, Sheets

1/6-6/6	as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 23
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1,3-5,14-22 (completely or partially)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☒ the claims, or said claims Nos. 1,4 (partially); 21,22 completely) are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. of inventions 2 and 3

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1,4,5,19,20 (partially) 2,6-11,13 (completely) (= invention 1) .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1,2,4-11,19,20
	No:	Claims	13
Inventive step (IS)	Yes:	Claims	1,2,4-11,19,20
	No:	Claims	13
Industrial applicability (IA)	Yes:	Claims	1,2,4-11,13,19,20
	No:	Claims	-

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on 24.05.2004

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item III.

1. In the present case, the set of claims was first considered with regard to Art. 5 and 6 (clarity and disclosure) before fulfilment of the requirement of Article 3(4)(iii) and 17(3)(a) (unity of invention) was examined.

2.1 Present claim 1 relates to an extremely large number of possible methods for producing a male sterile plant inter alia by decreasing the level of synthesis of 11- and/or 12-hydroxyjasmonate. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the method as detailed in claim 3.

2.2 Similarly, present claim 4 relates to an extremely large number of possible methods. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the methods involving genetic modification as claimed in claim 5.

2.3 Further, claims 21 and 22 refer to a composition for restoring normal anther development comprising at least one 11-/12-OHJA sulfotransferase inhibitor, without giving a technical characterization of such an inhibitor. Moreover, no such inhibitor is disclosed in the application. In consequence, the scope of said claims is ambiguous and vague and its subject-matter is not sufficiently disclosed and supported (Art. 5 and 6, PCT). No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the result to be achieved.

Re Item IV.

Requirement of unity:

The application lacks unity of inventions as required by Article 3(4)(iii) and 17(3)(a), PCT for the following reasons:

The inventions as defined in the enclosed International Search Report relate to various methods of producing male sterile plants and to compositions for restoring fertility.

The common concept underlying the present application is that those methods of producing male sterile plants and compositions for treating said plants all involve the modulation of enzyme activity, wherein the enzymes are involved in the plant jasmonate pathway and the use of a said compositions on said male sterile plants.

Methods for producing male sterile plants by modulating the activity of enzymes of the plant jasmonate pathway are already known in the art (please see e.g. WO9710703 page 4, line 28-page, 5, line 6; page 7, lines 3-10; page 8, line 22-page 10, line 2 or Park J.-H. et al, Plant J., 2002,31(1),1-12, especially page 3, column 3, paragraph 2-page 4, column 2, paragraph 4). Further compositions comprising jasmonate for restoring fertility are also known (e.g. WO9710703 page 10, line 13-page 11, line 5).

In light of this prior art the above mentioned common concept is not novel and the problem underlying the present application can be redefined as:

The provision of additional methods for producing male sterile plants by modulating the activity of enzymes of the plant jasmonate pathway and additional compositions for restoring fertility comprising jasmonate.

The methods identified in inventions 1 to 3 are different solutions to this problem.
Due to the fact that methods for producing male sterile plants by modulating the activity of enzymes of the plant jasmonate pathway and compositions for restoring fertility of such plants are known in the prior art and due to the fact that no other technical feature can be distinguished which, in the light of the prior art, could be regarded as a special technical

feature in the sense of Rule 13.2, PCT, there is no single general inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rule 13.1, PCT.

Consequently, the application lacks unity of invention and the different inventions are as formulated as follows:

a) Invention 1: 1,4,5,19,20 (partially); 2,6-11,13 (completely)

Methods for producing a male sterile plant involving increasing the endogenous activity of a plant hydroxyjasmonate sulfotransferase; plant transformation vectors comprising i.a. a nucleic acid having at least 50% homology to SEQ ID NO:1 or 2 or encoding an amino acid having at least 50% homology to SEQ ID NO:3 or 4; a genetically modified plant with elevated hydroxyjasmonate sulfotransferase levels.

b) Invention 2: 1,4,5,19,20 (partially); 3,12 (completely)

Methods for producing a male sterile plant involving decreasing the synthesis of a jasmonic acid 11-/12-hydroxylase and a genetically modified plant with decreased 11- or 12-hydroxyjasmonate levels.

c) Invention 3: 14-18 (completely);19,20 (partially)

Compositions comprising i.a. jasmonate and methods for using the same.

Re Item V.

The applicant's comments, filed with the letter dated 16.02.2005 regarding novelty, unity of inventions, lack of support and formal rejections with regard to claims 1-22 were taken into account for establishing the present report.

1. Novelty and inventive step (Art. 33(2)(3), PCT)

It is pointed out that this International Preliminary Report only refers to the claims of invention 1 as far as these claims have been searched (please see Item III).

The following document is referred to in this communication:

D1 : WO 01/02589 A (VARIN LUC ; GIDDA SATINDER (CA)) 11 January 2001 (2001-01-11)

1.1 Invention 1 of the present application relates to methods for producing male sterile plants by increasing the plant in vivo sulfonation of hydroxyjasmonate through overexpression of hydroxyjasmonate sulfotransferase. Further, genetically modified male sterile plants having elevated levels of 11- or 12- hydroxyjasmonate sulfotransferase are claimed.

1.2 D1 relates to methods for modulating flowering in plants inter alia by increasing hydroxyjasmonic acid sulfotransferase activity in plants (page 21, line 31-page 22, line 28; Exp. 2). D1 also discloses genetically modified plants, which overexpress

hydroxyjasmonic acid sulfotransferase and therefore contain higher levels 11- or 12-hydroxyjasmonate sulfotransferase than non-genetically modified plants (page 25, paragraphs 2 and 3; Table 1). Although it is not mentioned that said plants are male sterile, male sterility will be an intrinsic property of said plants. Consequently, subject-matter of present claim 13 lacks novelty according to Art. 33(2), PCT.

2. Remarks referring to the objection of lack of unity of inventions under item IV

In reply to the objection of lack of unity of inventions the applicant submitted arguments in favour of unity of subject-matter identified as inventions 1-3 (letter dated 16.02.2005). The technical feature linking claims of inventions 1-3 was identified as "the modulation of hydroxyjasmonate sulfotransferase".
This feature is however not suited to link inventions 1-3 because this feature is not present in invention 2. Invention 2 relates to decreasing the activity of a jasmonic acid 11-/12-hydroxylase, but not to a modulation of hydroxyjasmonate sulfotransferase. Hence the above stated technical feature is not common to all three inventions and therefore cannot be the basis of a "single general inventive concept" according to Ruel 13.1, PCT.
Further, it is pointed out that compositions comprising jasmonate for restoring fertility as claimed in invention 3 are apparently known in the prior art (see paragraph 4 of Item IV).
Consequently, the objection of lack of unity is maintained (Art. 3(4)(iii), Art. 17(3)(a), PCT).

Re Item VIII.

1.1 For reasons of consistency and clarity only one of the terms
a) "jasmonic acid" or jasmonate",

b) "hydroxyjasmonic acid" or hydroxyjasmonate", and

c) "11-or 12-hydroxyjasmonic acidsulfotransferase" or "11-or 12-hydroxyjasmonic acid sulfotransferase" (claim 13)

should be used throughout the claims and the description (Art. 5, 6, PCT).

1.2 Furthermore, the applicant's statement in the letter dated 16.02.2005 (page 2, paragraph 2) that the term "jasmonate" should be interpreted as referring exclusively to "12-hydroxyjasmonate", leaves the reader in doubt as to the meaning of the term, so that the claims and description referring to "jasmonate" cannot be regarded as clear and thus contravene Art. 5, 6, PCT.

CLAIMS

1. A method for producing a male sterile plant characterized in that said method comprises the step of decreasing the level of 11-and/or 12-hydroxyjasmonate by increasing in said plant the level of in-vivo sulfonation of hydroxyjasmonates or decreasing the level of synthesis of 11-and/or 12-hydroxyjasmonate.

2. The method of claim 1, characterized in that the level of in-vivo sulfonation of hydroxyjasmonates is increased by increasing in said plant the endogenous activity of a hydroxyjasmonatesulfotransferase.

3. The method of claim 1, characterized in that the level of synthesis of 11-and/or 12-hydroxyjasmonate is decreased by decreasing in said plant the activity of a jasmonic acid 11-/12-hydroxylase.

4. The method according to any one of claims 1 to 3, characterized in that the increasing of the level of in-vivo sulfonation of hydroxyjasmonates or the decreasing of the level of synthesis of 11-and/or 12-hydroxyjasmonate is achieved by a process selected from the group consisting of genetic modification of said plant, radiation mutagenesis of said plant, chemical mutagenesis of said plant and selection of natural mutants.

5. The method of claim 4, characterized in that said method consists of genetic modification.

6. The method of claim 5, characterized in that the endogenous activity of the sulfotransferase is increased by stimulating the expression of at least one gene selected from the group consisting of: *AtST2a*, *AtST2b*, functional homologues thereof having at least 50% homology with SEQ ID no.1 or SEQ ID no. 2 and a nucleic acid encoding for an amino acid sequence

having at least 50% homology with amino acid sequence of SEQ ID no. 3 or SEQ ID no. 4.

7. A method for producing a male sterile plant, characterized in that said method comprises:
   - introducing into a cell of a suitable plant an exogenous nucleic acid molecule via a vector capable of facilitating transfer and expression of an exogenous nucleic acid into an isolated cell and/or facilitating integration of an exogenous nucleic acid into genome of said cell, said vector comprises at least one promoter sequence, one enhancer sequence and one exogenous nucleic acid sequence, said promoter being a constitutive expression promoter or an inducible promoter, said exogenous nucleic acid being selected from the group consisting of nucleic acid sequence having at least 50% homology with SEQID no. 1 or SEQ ID no. 2 and a nucleic acid encoding for an amino acid sequence having at least 50% homology with amino acid sequence of SEQ ID no. 3 or SEQ ID no. 4;
   - regenerating a transgenic plant from said cell ; and where necessary
   - growing the transgenic plant for a time and under conditions sufficient to permit expression of the exogenous nucleic acid sequence and thereby stimulating expression of the hydroxyjasmonic acid sulfotransferase.

8. The method of claim 7, characterized in that the inducible promoter of the vector is an ethanol-inducible promoter or a glucocorticoid-inducible promoter.

9. The method of claim 7, characterized in that the constitutive expression promoter of the vector is an ubiquitin promoter.

10. The method of claim 7, characterized in that the promoter of the vector isCaMV 35S promoter.

11. The method of claim 7, characterized in that the enhancer of the vector is an AMV translational enhancer.

12. A genetically modified male sterile plant, characterized in that the endogenous level of 11-or 12-hydroxyjasmonate in said genetically modified male sterile plant is lower than the endogenous level of 11-or 12-hydroxyjasmonate in a non genetically modified plant.

13. A genetically modified male sterile plant, characterized in that the endogenous level of 11-or 12-hydroxyjasmonic acidsulfotransferase in said genetically modified male sterile plant is higher than the endogenous level of 11-or 12-hydroxyjasmonic acid sulfotransferase in a non genetically modified plant.

14. A composition for restoring normal anther development in a genetically modified male sterile plant, comprising at least one jasmonate and an acceptable carrier.

15. The composition of claim 14, characterized in that the jasmonate is selected from the group consisting of: 11-hydroxyjasmonic acid, 12-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, glucoside of 11-hydroxymethyljasmonic acid, 12-hydroxymethyljasmonic acid and glucoside of 12-hydroxymethyljasmonic acid.

16. The composition of claim 14, characterized in that the composition contains 100 μM of 12-hydroxyjasmonic acid or 50 μM methyl-jasmonic acid.

17. The composition of claim 16, characterized in that the composition further contains "TWEEN20".

18. The composition of claim 17, characterized in that the composition containsTWEEN20™ in the concentration of 0.05 weight percent of the total volume of said composition.

19. A method for restoring normal anther development in a genetically modified male sterile plant as defined in any one of claims 12 or 13, comprising a step of applying on male sterile flowers of said plant a composition as defined in any one of claims 14 to 18.

20. The method of claim 19, characterized in that the step of applying said composition on male sterile flowers of said plant is achieved by soaking male sterile flowers in said composition for two minutes daily starting 7 days before appearance of a first flower bud and ending 7 days after appearance of a first flower.

21. A composition for restoring normal anther development in a genetically modified male sterile plant, comprising at least one 11-/12-OHJA sulfotransferase inhibitor and an acceptable carrier.

22. A method for restoring normal anther development in a genetically modified male sterile plant as defined in any one of claims 13 or 14, comprising a step of applying on male sterile flowers of said plant a composition as defined in claim 21.